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# CHARACTERISTICS OF INFLUX AND NET FLUX OF AMINO ACIDS IN *MYTILUS CALIFORNIANUS*

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Influx of $^{14}$C-labelled amino acids across the epidermis has been observed in a number of soft-bodied marine invertebrates (see Stephens, 1972, for a review). Such influx in molluscs has been reported by several workers (recent examples include Anderson and Bedford, 1973; Bamford and Campbell, 1976; Bamford and McCrea, 1975; Péquignat, 1973; Stewart and Bamford, 1975; Wright, Johnson and Crowe, 1975). Some of these studies employed intact organisms, while others reported influx of amino acids into isolated gills or gill discs. In no case was net flux examined. The only report of net influx of amino acids in a mollusc is that of Stephens and Schinske (1961). They employed high external concentrations for most of their work (2 mM) but observed net influx in the clam, *Spisula,* from ambient glycine concentrations of 20 μM. However, they did not attempt to follow net influx closely and describe its kinetics, nor did they compare net flux with influx.

Simultaneous measurement of influx and net flux in lamellibranchs is of considerable interest for at least three reasons. First, it is net flux which is of interest in assessing the potential nutritional significance of trans-epidermal amino acid uptake for the organism. Several of the workers cited draw tentative conclusions based on the more or less clearly stated assumption that influx (their observed phenomenon) can be equated (sometimes with stated reservations) with net flux. This assumption may be approximately correct for sediment-dwelling forms. Stephens (1975) reports net influx of amino acids at ambient levels well below those found in the sediment environment. However, ambient concentrations of amino acids normally present in the water column are substantially lower. Hence influx cannot be assumed to reflect net flux at naturally occurring ambient concentrations for ciliary-mucoid filter feeders such as lamellibranchs.

The second reason for interest in net flux relates to studies of the mechanism of influx. Intracellular free amino acid pools are very large in marine invertebrates (typically of the order of $2–5 \times 10^{-1}$ M). In typical studies of influx, labelled amino acids are supplied at low ambient concentrations. Under such circumstances, if the labelled substrate were entering the organism by a process such as exchange diffusion, rapid influx of labelled material would be observed, even though accompanied by no flux or even by net efflux. Indeed, Johannes, Coward and Webb (1969) have proposed precisely this explanation to account for observations of influx of labelled amino acids, based on their studies of influx and net flux in an ectoparasitic flatworm, *Bdelloura.* They did not directly examine the predictable consequences of an exchange diffusion mechanism. However, this can readily be done if influx and net flux are monitored simultaneously under a suitable range of conditions.

Thirdly, Stewart and Bamford (1975) and Wright (1976) report evidence that influx of amino acids in molluscs is sodium-sensitive. Sodium dependence has yet to be demonstrated for transepidermal amino acid transport in any marine invertebrate, including molluscs. One of the key observations which is considered to be convincing evidence of sodium-coupled or sodium-dependent transport of an organic solute is demonstration of sodium-coupled efflux from cell to medium (Schultz and Curran, 1970). Simultaneous studies of influx and net flux as a function of external sodium permit such observations.

Studies of net flux of amino acids have been facilitated by the recent introduction of the reagent, fluorescamine (Udenfriend, Stein, Böhlen, Dairman, Leimgruber and Weigle, 1972). Fluorescamine forms a fluorescent product with primary amines in aqueous solution permitting simple and routine determination of amino acid concentrations as low as $10^{-7}$ M. North (1975) and Stephens (1975) describe procedures for determining primary amines in sea water using this reagent.

In the present report, the initial report of Wright *et al.* (1975) on the kinetics of amino acid influx in isolated gills of *Mytilus* is extended and refined. Simultaneous measurements of influx and net flux in this organism are also reported, and the implications of such measurements for hypotheses concerning the nutritional significance of amino acid influx and the mechanism of this influx are described.

## Materials and Methods

Specimens of *Mytilus californianus* were collected from the open coast of southern California near Laguna Beach. Animals were kept at 13° C in aquaria in aerated sea water. Preliminary studies showed no difference in experimental results between freshly collected mussels and those maintained in aquaria for eight weeks. Animals were usually used within four weeks of collection.

All experiments were conducted in artificial sea water (ASW, MBL) prepared with reagent grade salts and distilled water according to Cavanaugh (1956). When sodium-free incubation media were used, choline chloride or LiCl were substituted for NaCl, and $KHCO_3$ for $NaHCO_3$. These solutions were isosmotic to ASW, MBL (salinity = 31‰), and they had a pH of 7.8–8.0.

Mussels were opened by cutting the posterior adductor muscle. The four demibranchs were removed, placed on a sheet of aluminum foil and discs of gill tissue cut with a 1.0 cm stainless steel cork borer. These discs of gill tissue had a mean wet weight of $32.2 \pm 0.7$ (s.e.) mg, and a mean dry weight of $5.0 \pm 0.1$ mg. Gill discs were held in MBL at 16° C for thirty minutes prior to observations. Discs from at least three different mussels were used in all influx experiments. The procedure for determination of influx rates was as follows. Nine discs of gill tissue were introduced into 200 ml of aerated MBL containing cycloleucine (1-aminocyclopentane-1-carboxylic acid) or glycine. Solutions contained approximately 0.05 $\mu$Ci/ml of (U-$^{14}$C)cycloleucine (New England Nuclear) or (U-$^{14}$C) glycine (Schwartz-Mann) and sufficient $^{12}$C substrate to give final concentrations ranging from 2 $\mu$M to 5 mM. Three discs of tissue were removed from the solution at 3, 6 and 9 minutes, rinsed rapidly (5–10 seconds) in two successive 0° C MBL rinses, and blotted gently on filter paper. Individual discs were placed in scintillation vials containing 1 ml of 0.1 N $HNO_3$. After extraction for at least twelve hours, a toluene-based scintillation cocktail was added and the extracted discs were

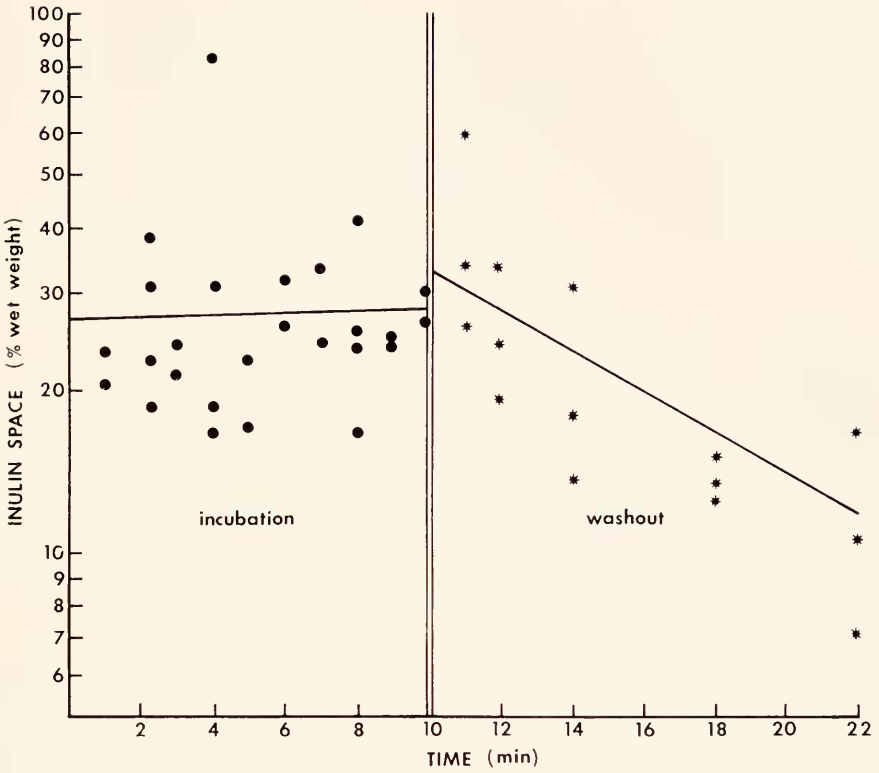


FIGURE 1. Labelling and washout of gill discs with $^{14}$C-inulin. Labelling is expressed as inulin space in units of percentage of tissue wet weight. Each point represents radioactivity associated with a single gill disc after rinsing and blotting. Lines are least squares regressions.

counted using a Beckman CPM-100 scintillation counter. Initial and final samples of the medium were taken and counted for each experiment. The volume of the medium was sufficient so that no significant decrease in radioactivity was observed.

Extracellular space was determined using (U-$^{14}$C)inulin. A solution containing trace amounts of labelled inulin in MBL was prepared. After exposure to this solution for various times, sets of tissue discs were rinsed, blotted and extracted as described above. To determine the time course of washout of inulin, tissue discs were incubated in the labelled inulin solution for ten minutes, transferred to a large volume of MBL and removed at intervals. Radioactivity was determined as described.

Simultaneous measurements of influx and net flux of glycine in intact mussels were performed as follows. Mussels were cleaned with a wire brush and kept at 16° C in MBL containing streptomycin (200 mg/liter), chloramphenicol (100 mg/liter) and penicillin (500,000 I.U./liter) to reduce microbial activity. After 24 hours, animals were rinsed in MBL and placed individually in 500 ml of MBL containing 0.05 $\mu$Ci/ml $^{14}$C-glycine plus unlabelled substrate. Samples of the medium were taken at intervals after introducing the animal. One set of samples

was counted for radioactivity. Another set was used for determination of primary amines as follows. A 0.5 ml sample was buffered to pH 9.2 using 0.5 ml of 0.2 M borate buffer and mixed vigorously with 0.5 ml of acetone containing fluorescamine (200 mg/liter). After addition of 2.0 ml of distilled water, fluorescence at 480 nm with excitation at 390 nm was measured using a Turner fluorometer (filters were used to approximate excitation and emission frequencies). A set of glycine standards was measured with each set of experimental determinations. Primary amine concentrations are expressed in glycine-equivalent concentration units.

## Results

### *Inulin space*

When gill discs are exposed to $^{14}$C-inulin, labelling is complete at the end of one minute and remains constant thereafter at approximately 30% of the wet weight of the tissue. This inulin space is interpreted to include rapidly labelled extracellular space together with the portion of the medium adhering to the gill discs after rinsing and blotting. When discs labelled with $^{14}$C-inulin are placed in a large volume of MBL, radioactivity disappears exponentially with a half time of approximately seven minutes (Fig. 1). The exponential character of the washout is consistent with the hypothesis that adherent medium and extracellular tissue space behave as a single compartment.

The influence of the presence of this rapidly labelled extracellular compartment on determinations of influx rates of organic substrates is illustrated in Figure 2. In the upper portion of Figure 2, the results of exposure of gill discs to $^{14}$C-cycloleucine at an ambient concentration of 50 $\mu$M is presented. Uptake is linear with time. A least squares regression line through the observed points intercepts the y-axis close to the point predicted by the inulin space. At this low concentration, the labelling of the inulin space (*i.e.*, $^{14}$C-cycloleucine counted as a result of its presence in the extracellular space) contributes little to the total tissue radioactivity. It represents about 3% of the total radioactivity associated with the tissue after a ten-minute exposure to the solution. Washout of radioacivity when the tissue is placed in MBL is imperceptible.

The lower portion of Figure 2 presents similar observations, but cycloleucine is supplied at an ambient concentration of 5 mM. Rapid labelling of the inulin space accounts for a very significant fraction of the total radioactivity associated with the tissue. Again, uptake is linear and again a regression line intercepts the y-axis at the point predicted by measurement of the rapidly labelled extracellular compartment. However, although influx is more rapid at the higher ambient concentration, the carrier mediating influx is fully saturated and the total influx during the brief observation period is a small fraction of ambient concentration. Therefore, radioactivity associated with the rapidly labelled compartment accounts for approximately 66% of the total radioactivity present in the tissue at the end of ten minutes. The fact that a large fraction of the radioactivity was present in the extracellular space is also indicated by the exponential washout shown in the lower portion of Figure 2. If the rate of cycloleucine influx were calculated from the total amount of radioactivity associated with the tissue after a five-minute incubation, the rate would be overestimated by approximately 350%.

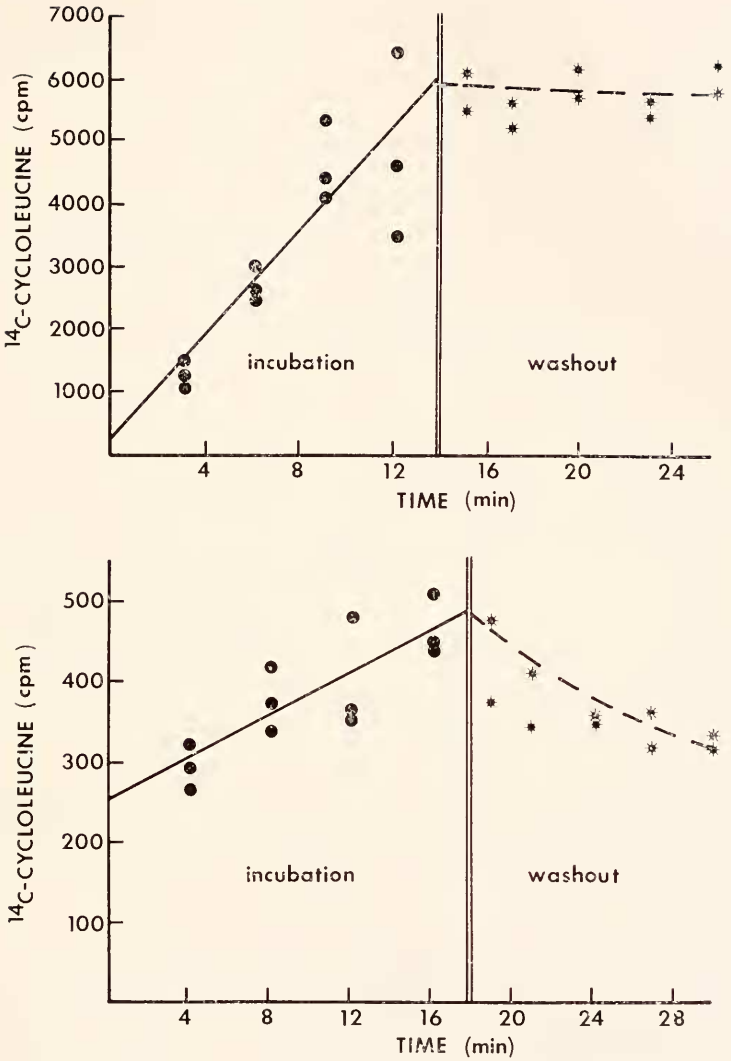


FIGURE 2. The time course of uptake and washout of $^{14}$C-cycloleucine into gill discs incubated in two different concentrations of $^{12}$C-cycloleucine. Each point is the radioactivity from a single gill disc. The solid lines were fit by the method of least squares; the dashed lines are the rates of washout predicted by the data in Figure 1. The y-intercepts predicted by the data in Figure 1 are 198 cpm and 255 cpm for the upper and lower graphs, respectively.

*Kinetics of cycloleucine and glycine influx*

The effect of increasing concentration of cycloleucine in the medium on influx of cycloleucine is shown in Figure 3. At low concentrations the influx of cycloleucine ($J^i$) increases in a nonlinear fashion with increasing concentration in the medium (S). At high concentrations, $J^i$ increases linearly with S. However,

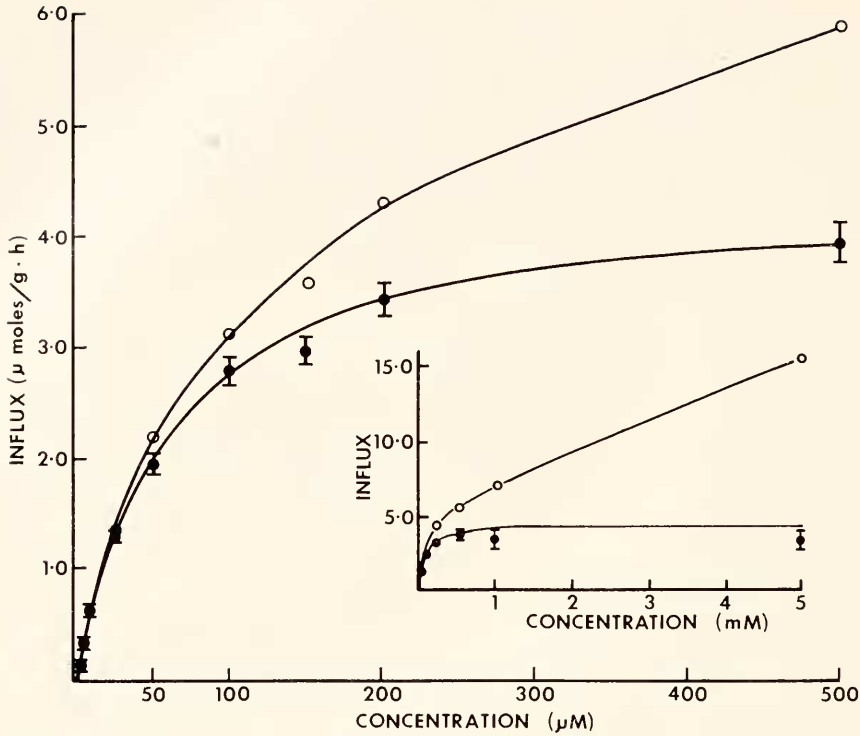


FIGURE 3. The influx rate of $^{14}$C-cycloleucine into gill tissue as a function of increasing concentration of cycloleucine. Open circles are mean values for cycloleucine influx uncorrected for the presence of inulin space; closed circles are values for influx corrected for inulin space. Units of influx in the inset are $\mu$moles/(g·hr); concentration is in mM. Bars signify $\pm$ 1 s.e. (n for each point ranges from 12 to 54).

when the data are corrected for $^{14}$C-cycloleucine present in the rapidly labelled inulin space, this second linear component virtually disappears. The slope of $J^i$ as a function of S at high concentrations is nearly zero. Cycloleucine influx based on corrected data is fully described by the Michaelis-Menten equation: $[J^i = (J^i_{max}(S))/(K_t + (S))]$, where $J^i_{max}$ is the maximum rate of influx and $K_t$ is the ambient concentration at which influx is half the maximum rate.

The constants $J^i_{max}$ and $K_t$ for cycloleucine influx were determined graphically from a plot of $(S)/J^i$ against $(S)$. $J^i_{max}$ is 4.5 $\mu$M/(g·hr) and $K_t$ is 63 $\mu$M. Similar data were obtained for glycine influx. When corrected for the labelling of inulin space, the constants for glycine were determined to be $J^i_{max} = 4.6$ $\mu$M/(g·hr) and $K_t = 34$ $\mu$M. There was no residual "passive" component perceptible in the influx data for either cycloleucine or glycine after correcting for inulin space. $J^i_{max}$ for the two substrates is not significantly different, suggesting that cycloleucine and glycine may enter by the same carrier-mediated pathway. This suggestion is also consistent with the inhibition data presented by Wright *et al.* (1975).

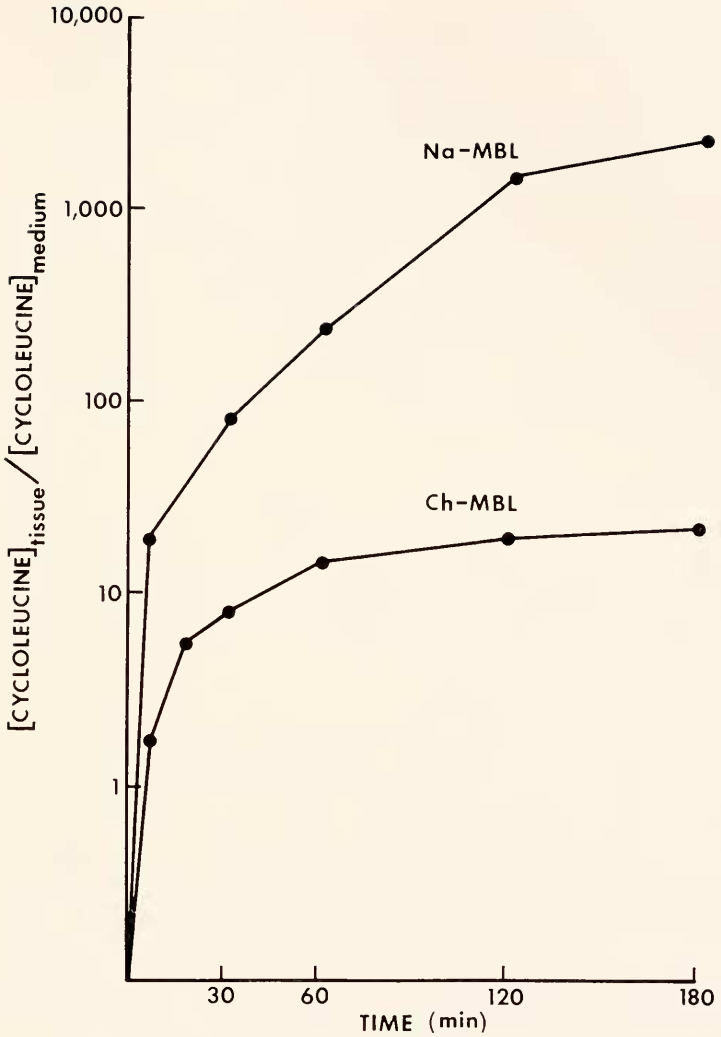


FIGURE 4. The tissue-to-medium concentration gradient of cycloleucine developed by isolated gill tissue in MBL (Na-MBL) or MBL with choline replacing sodium (Ch-MBL) as a function of time; at each point, n = 3.

*Effect of external sodium on transport of cycloleucine*

Influx of cycloleucine into gill discs was reduced to 10% or 20% of control values when sodium in the medium was replaced by lithium or choline respectively. Cycloleucine concentration was 50 $\mu$M. This inhibition of influx in the absence of sodium is at least partially reversible. When gill discs exposed to sodium-free MBL for one hour were washed in regular MBL for fifteen minutes, the rate of cycloleucine influx returned to 77% of control values.

Figure 4 presents data concerning steady state concentrations of cycloleucine present in gill tissue after prolonged exposure to a small volume of MBL containing 50 μM cycloleucine. Tissue concentration is calculated based on wet weight excluding inulin space. Since cycloleucine is not metabolized and is not normally present in *Mytilus*, concentration is simply related to radioactivity and specific activity. In the presence of sodium (425 mEq/liter = MBL) ambient cycloleucine decreases in our system (10 ml of medium) to approximately 10 μM, while tissue concentration increases over the course of three hours to approximately 20 mM. When sodium is replaced in the medium by choline, the medium concentration does not fall perceptibly below 50 μM while tissue concentration increases to approximately 1 mM. Similar observations using glycine as a substrate produce nominal concentration ratios based on radioactivity of approximately 15,000 : 1 in the presence of sodium; 10 : 1 in the absence of sodium. However, glycine is normally present in the intracellular free amino acid pool and is metabolized by the gill tissue so such figures cannot be interpreted simply.

*Influx of glycine and net flux of primary amines*

The following observations were made on intact mussels. Glycine was used as the substrate, both because it is a major constituent in naturally occurring free amino acids in sea water and because the specific fluorescence of the product of its reaction with fluorescamine is suitably high (North, 1975). Cycloleucine produces a weakly fluorescent product with fluorescamine so it was not suitable for these observations.

Figure 5 presents data for influx of $^{14}$C-glycine based on disappearance of radioactivity from the medium together with data for net flux of primary amines based on the fluorescamine procedure. Samples of the medium were taken at the same time for the two procedures and various initial concentrations of glycine were employed as indicated in the figure. At high ambient concentrations (20–100 μM) influx of $^{14}$C-glycine corresponds closely to net influx of primary amines. At the other extreme, primary amines appeared in the medium when mussels were placed in MBL containing no amino acid and reached a steady state concentration of 2–4 μM which was stable for at least 24 hours. At ambient concentrations of $^{14}$C-glycine below 20 μM, influx of glycine estimated from radioactivity is consistently more rapid than net influx of primary amines estimated from fluorescamine determinations. This discrepancy becomes more pronounced as $^{14}$C-glycine concentrations decrease.

This relation is expressed graphically in Figure 6. The ratio $^{14}$C-glycine/primary amine is plotted against glycine concentration as estimated by radioactivity of the solution. At high concentrations that ratio approaches unity. At low concentrations, the efflux of the unknown primary amines becomes increasingly significant relative to $^{14}$C-glycine influx and the ratio declines. Data in Figure 6 include those presented in Figure 5, as well as additional data from similar experiments. The line in Figure 6 is drawn to fit the equation $y = x/(x + 1.06)$, and represents the assumption that efflux is constant at all concentrations of glycine in the medium. The mean difference in concentration between $^{14}$C-glycine and total primary amine is 1.06 μM in the ten cases observed where $^{14}$C-glycine concentration was reduced to less than 10 μM.

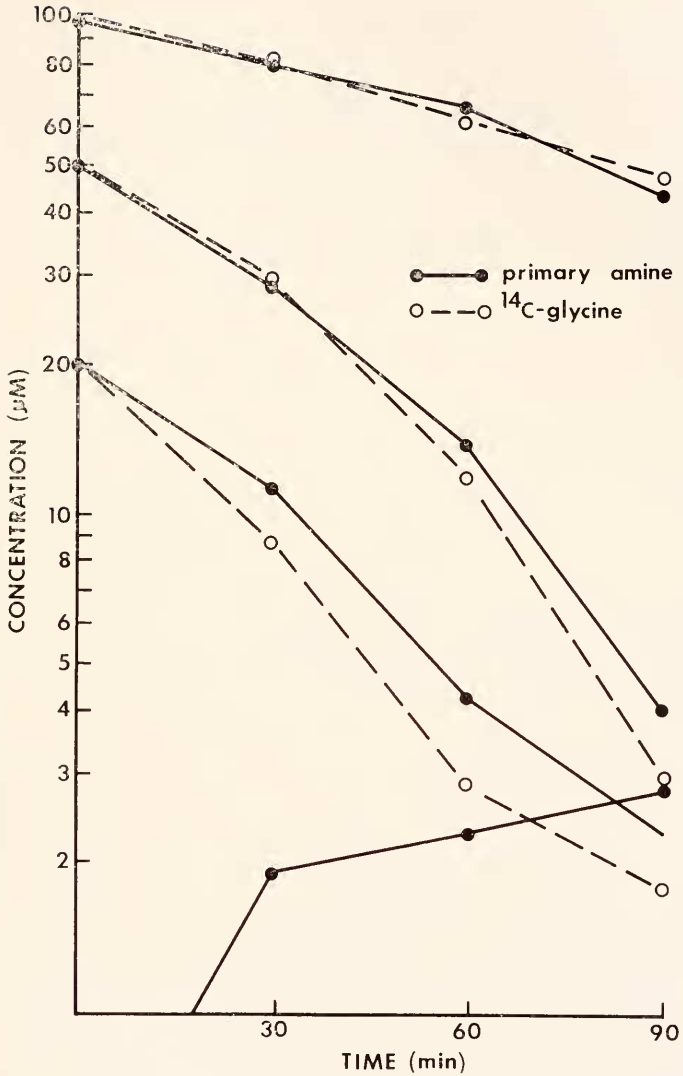


FIGURE 5. The clearance of $^{14}$C-glycine and total primary amine from MBL by intact *Mytilus* as a function of time. The volume of experimental medium in each experiment was 500 ml, and the initial concentrations of glycine were 100 μM, 50, 20, and 0. The closed circles are the concentrations of total primary amine measured as glycine equivalents using fluorescamine. The open circles are the concentrations of $^{14}$C-glycine. Each line represents the clearance by one mussel.

*Influx of glycine and net flux of primary amines: sodium effects*

Gill discs liberate primary amines into the medium resulting in a relatively high background of fluorescamine-positive material. This was a reason for choosing intact mussels for the preceding observations. However, the replacement of external

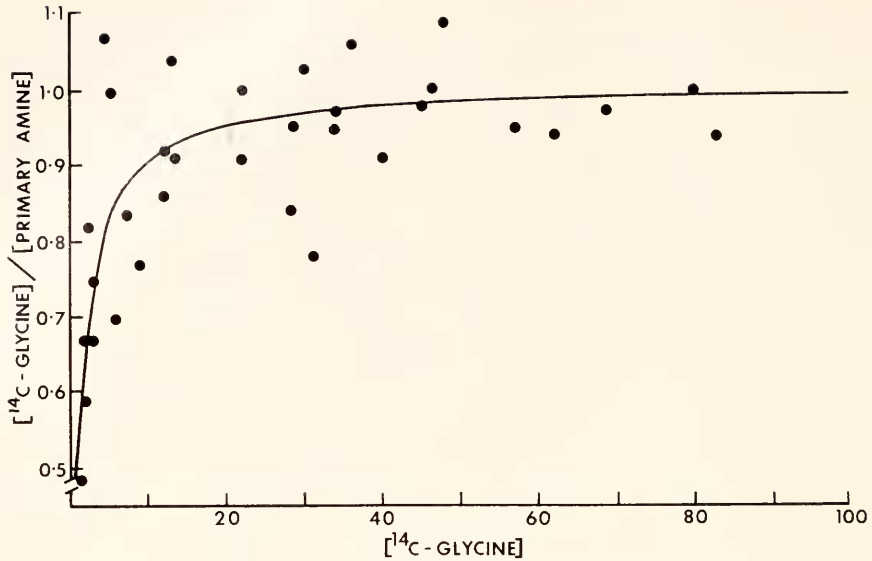


FIGURE 6. The ratio of the concentration in the medium of radioactive glycine to total primary amine measured in duplicate samples during clearance experiments plotted as a function of the radioactive glycine concentration. Data were taken from Figure 5 and similar experiments. The line fits the equation, $y = x/(x + 1.06)$, and expresses the assumption that efflux is constant at all concentrations of glycine in the medium.

sodium with other ions interferes with normal pumping activity of the lateral cilia. Therefore, it was necessary to use isolated discs of gill tissue to examine the influence of external sodium on the relation between influx and net flux.

Three discs were placed into each of a series of beakers containing 10 ml of MBL plus 10 $\mu$M $^{14}$C-glycine. A parallel set of tissue discs were placed into beakers containing 10 ml of MBL in which sodium had been replaced by choline, also containing 10 $\mu$M $^{14}$C-glycine. At prescribed times the tissue in a beaker was removed and samples of the medium taken. Figure 7 presents the results of following the level of radioactivity and the level of primary amines in such an experiment. Radioactivity in the MBL medium declined to very low levels in the course of the experiment (equivalent to about 0.2 $\mu$M at 4 hours); primary amines fluctuated between 8 and 16 $\mu$M expressed as glycine equivalents with no clear trend with time. When sodium was replaced with choline, radioactivity decreased very slightly over the four-hour period. Primary amines in the medium increased steadily reaching 37 $\mu$M as glycine equivalents at the end of four hours.

## DISCUSSION

The data presented here show that the influx of cycloleucine and glycine is carrier-mediated. The *in vitro* gill preparation yields uptake data that are, however, easily misinterpreted as being indicative of a two component transport process: at low concentrations ($< 20$ $\mu$M), uptake appears to involve a high affinity saturable system, while at high concentrations ($> 20$ $\mu$M), either a low

affinity system or passive diffusion appears to become increasingly important. However, when the data are corrected for the presence of the inulin space, influx appears to be by a single component, fully saturable system, which follows Michaelis-Menten type kinetics. No evidence was found to support postulation of a second, low-affinity carrier system. The close agreement of $J^{i}_{max}$ for the two substrates suggests that glycine and cycloleucine enter *via* the same pathway(s).

Passive diffusion can play no significant role in the influx of amino acids. *Mytilus* and other bivalves maintain high steady-state concentrations of free amino acids in their tissues. If the gill membrane had permeability properties such that passive diffusion could occur from external concentrations in the 100 $\mu$M range

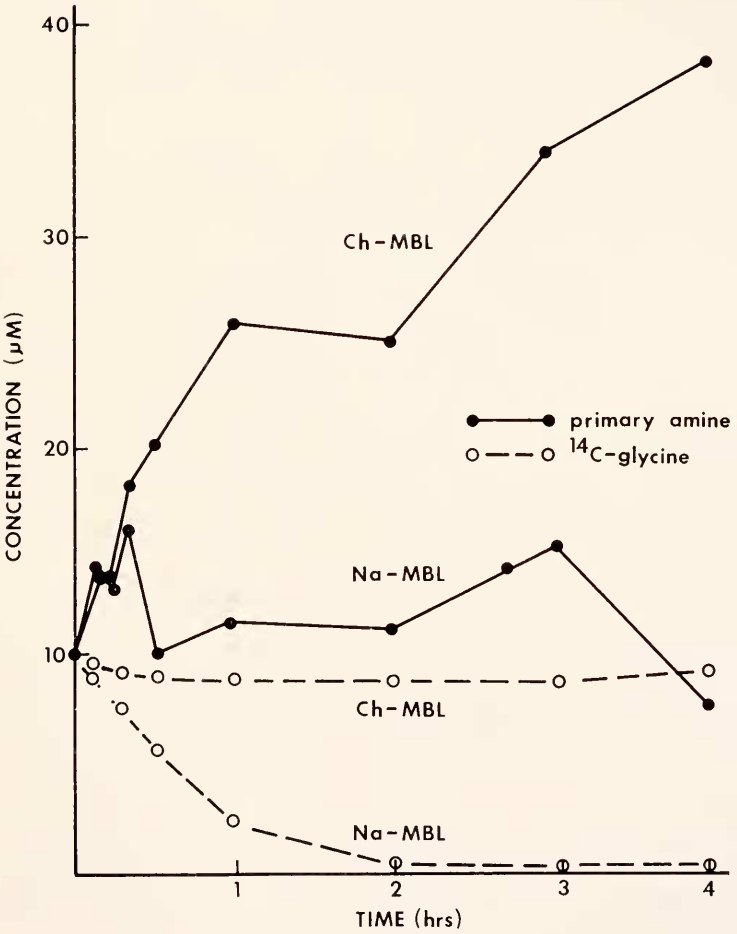


FIGURE 7. The change in medium concentration of $^{14}$C-glycine and total primary amine resulting from the incubation of isolated gill tissue in either Na-MBL or Ch-MBL as a function of time. The data obtained at each time interval represent the measurements of medium concentration in separate parallel experiments. Duplicate samples were taken for the determination of radioactivity and total primary amine.

at rates falsely implied by the uncorrected data, the symmetrical passive efflux of amino acid from the approximately half molar intracellular free amino acid pool would make the maintenance of such pools energetically impossible.

The kinetic parameters calculated from the data on influx rates for cycloleucine and glycine are similar to the $K_t$'s and $V_{max}$'s reported for uptake of several neutral amino acids by other preparations of isolated bivalve gill (Wright, *et al.*, 1975 on *Mytilus californianus;* Bamford and Campbell, 1976 on *M. edulis;* Bamford and McCrea, 1975 on *Cerastoderma edule;* Stewart and Bamford, 1975 on *Mya arenaria;* Anderson and Bedford, 1973 on *Rangia cuneata*). These investigators reported $K_t$'s ranging from 12 $\mu$M to 250 $\mu$M, and $V_{max}$'s from approximately 0.5 $\mu$M/(g·hr) to 35 $\mu$M/(g·hr). However, none of the above workers corrected their data for the possible presence of a rapidly-labelled extracellular compartment such as the one noted in the present study. The importance of such a correction for the interpretation of uptake data makes the kinetic parameters of these carrier studies suspect. We wish to replace the values reported earlier (Wright, *et al.*, 1975) with those in the present work.

The data summarized in Figures 5 and 6 indicate that the disapparance of radioactivity from the medium as a result of glycine influx into *Mytilus* accurately represents net influx of glycine into the organism at concentrations greater than 20 $\mu$M. This does not exclude the possibility that glycine influx and the net influx observed may not be accompanied by efflux of small amounts of unidentified primary amines. Such an efflux is the simplest explanation of the discrepancy observed between glycine influx and net influx at lower ambient concentrations. If efflux also occurs at higher concentrations, it would be expected to elevate primary amine determinations by a few parts per hundred at the higher concentrations employed in this study; this is about the range of analytical error for the procedure.

The close parallel of influx and net flux at high concentrations excludes simple exchange diffusion as a mechanism to account for influx of $^{14}$C-glycine. That hypothesis predicts a stable ambient concentration of primary amines at the initial level accompanied by an exponential decrease in radioactivity, which is contrary to the data presented here.

Primary amines in the medium are reduced by *Mytilus* to levels ranging from 2–5 $\mu$M (as glycine equivalents), the concentration range in which steady-state is achieved. The steady-state seems independent of the initial level of amino acid supplied. At concentrations greater than 2–5 $\mu$M, net influx is observed until steady-state is reached. At concentrations lower than this, steady-state is achieved by net efflux. It should be emphasized that the primary amines which appear in the medium are at present unidentified. Expression of their concentration as glycine-equivalents is arbitrary. The specific fluorescence of reaction products of primary amines with fluorescamine varies; as noted, cycloleucine produces a weakly fluorescent product; the products formed with polybasic amino acids fluoresce more strongly than glycine. The compound(s) which appear in the medium are currently being studied. With respect to the mechanism of efflux, simple exchange diffusion can be excluded, but further conclusions cannot be drawn.

These observations do not permit definite conclusions about the role of transepidermal amino acid uptake in the nutrition of *Mytilus*. If the animal experiences ambient concentrations greater than a few micromoles/liter in its environment,

net influx seems likely. This is much higher than the levels reported for free amino acids in the water column which range from 0.2 to 2 micromolar. Therefore, mussels presumably encounter sea water passing across their gills which is lower in amino acid content than the steady-state ambient concentration reported here. Despite that fact, the possibility of net influx from amino acid concentrations in the micromolar range cannot be excluded on the basis of the present work. If no change in the composition of primary amine occurred in our closed system, the steady-state level observed would be a measure of the capacity of *Mytilus* to clear primary amines from micromolar concentrations. However, there is no reason to suspect that this is the case. In an open system either net influx or efflux could occur. The direction of net movement of amino acid depends on the ratio of the rate of acquisition of primary amine(s) (naturally occurring) to the loss of primary amines(s) (from the gill) in a single pass of incurrent water across the gills.

Influx of $^{14}$C-glycine in gill discs of *Mytilus* is inhibited when external sodium is replaced by choline or lithium. This agrees with the observation reported by Stewart and Bamford (1975) of reduced rates of $^{14}$C-alanine influx into the isolated gills of the soft-shelled clam, *Mya arenaria*, in the absence of external sodium. Sensitivity to external sodium has been reported for many nonelectrolyte transport systems (reviewed by Schultz and Curran, 1970). The observation of a relation between external sodium and the rate of influx for an organic solute is an initial step in establishing the occurrence of sodium-coupled cotransport, and is consistent with the presence of such a system. However, it must be accompanied by additional observations before a sodium-coupled cotransport mechanism can be considered to be established. This has not been done for transepidermal amino acid transport in any marine invertebrate.

The present report of efflux of primary amines when sodium is replaced in the medium (Fig. 7) is also consistent with the existence of sodium-coupled cotransport in *Mytilus* gill. One of the key predictions made by sodium-coupled models of transport of organic solutes is that the organic solute should be translocated from the cell in circumstances where internal sodium exceeds external sodium (Schultz and Curran, 1970). The primary amines which appear in the medium bathing gill discs when external sodium is replaced with choline have not been identified. Therefore detailed discussion is not justified. However, it can be noted that if the primary amines are interpreted as free amino acids, the rate of efflux shown in the data of Figure 7 is approximately 20% of the $J^{i}_{max}$ for glycine and cycloleucine. This is an unreliable figure for an additional reason; sodium may affect $J_{max}$, and intracellular sodium has not been measured. Nevertheless, efflux under such conditions is consistent with a sodium-coupled transport system for amino acids.

Despite the support offered by the present observations for the existence of a sodium-coupled cotransport system, these data cast doubt on the ability of such a system to account fully for amino acid transport in the gill of *Mytilus*. First, there is apparent concentrative transport of cycloleucine in the absence of external sodium (Fig. 4). This is contrary to the prediction based on a sodium-coupled model. The data seem unambiguous, since cycloleucine is neither normally present in *Mytilus* nor is it metabolized. It is possible to frame an explanation based on hetero-exchange diffusion, with evidence for efflux into sodium-free medium. If

this were coupled to an exchange entry of cycloleucine, apparent concentrative transport would be observed. However, evidence indicates exchange diffusion does not normally occur, and it would not be predicted for a sodium-coupled model in the absence of external sodium.

The second *caveat* concerning a sodium-coupled mechanism as a complete explanation for these data is based on thermodynamic considerations. The basic feature of mechanisms for transport of organic solutes coupled to sodium transport is the stipulation that at least part of the energy necessary for active transport of the organic solute is not supplied by direct metabolic energy coupling but by the sodium concentration difference between the external solution and cytoplasm, *via* a coupled transport of sodium and the organic solute. Jacquez and Schafer (1969) formulated a series of equations to compare energy expenditure available from the sodium electrochemical potential gradient. The model for the coupling of the fluxes of amino acid and sodium most often proposed in conjunction with the sodium-gradient hypothesis involves the electrogenic 1 : 1 cotransport of sodium and amino acid. The maximum energy available in the sodium gradient in this case is: $E_{Na} = RT \ln[(Na^+)_e/(Na^+)_i] - FV$, where $(Na^+)_e$ is the extracellular concentration of sodium, $(Na^+)_i$ is the intracellular concentration of sodium, F is the Faraday constant (23 cal/mole-mV) and V is the membrane potential in mV (outside taken as zero potential). The variables are either known or can be estimated for *M. californianus* gill tissue. $(Na^+)_e$ is 425 mEq/liter in MBL (Cavanaugh, 1956). Potts (1958) reported that the sodium concentration in the sarcoplasm of several muscle types from *M. edulis* was approximately 80 mEq/liter. Murakami and Takahashi (1975) found a more or less steady resting potential of about 60 mV (inside negative) in the lateral cells from isolated gills of *M. edulis*. Using these figures, the potential energy available from the electrochemical gradient of sodium is approximately 2340 cal/mole. The energy required to maintain the amino acid gradient is: $E_{aa} = RT \ln[(aa)_i/(aa)_e]$, where $(aa)_i$ is the intracellular concentration of amino acid, and $(aa)_e$ is the extracellular concentration at steady state. Provided that coupling of the flows of sodium and amino acid were 100% efficient, the potential energy from the sodium gradient could account for the maintenance of a chemical gradient of amino acid of approximately 60 : 1.

This calculation is based on what may be overgenerous assumptions. Geck and Heinz (1976) argue that although an electrical potential difference is thermodynamically equivalent to a chemical concentration difference, in many cases it is less effective in supplying energy for sodium-coupled transport of organic solutes. In any case, the energy supplied by the electrogenic sodium gradient is inadequate to account for an observed steady state concentration gradient of cycloleucine of 2000 : 1 (Fig. 4).

Thus these data both provide additional support for the presence of a sodium-coupled mechanism for transport of amino acids in *Mytilus* gill and cast doubt on the adequacy of such a mechanism to provide a full explanation for this transepidermal transport.

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## Summary

1. Intact mussels and an *in vitro* preparation of isolated gill tissue were employed to study characteristics of the influx and net flux of amino acids in *Mytilus californianus*.

2. The kinetics of influx of $^{14}$C-labelled amino acids were complicated by the presence of a rapidly labelled extracellular compartment.

3. Correction of influx data for the extracellular compartment revealed influx of $^{14}$C-cycloleucine and $^{14}$C-glycine to be mediated by a transport mechanism adequately described by Michaelis-Menten kinetics. Passive diffusion plays no significant role in influx.

4. Influx and net flux of glycine into intact *Mytilus* were examined. From high concentrations ($> 20$ $\mu$M) the influx of $^{14}$C-glycine was equivalent to the net influx of primary amine as determined by fluorescamine. At low ambient concentrations ($< 20$ $\mu$M), influx of $^{14}$C-glycine occurred more rapidly than net influx as determined by fluorescamine. The data suggest that influx of labelled substrate is accompanied by efflux of unknown primary amine(s). In the absence of labelled substrate, efflux continues until a steady-state concentration of 2–5 $\mu$M in the medium is achieved.

5. The rate of influx of $^{14}$C-cycloleucine into isolated gill tissue, and the concentration gradient which can be developed by gill tissue are reduced when sodium is replaced in the medium.

6. The efflux of primary amines from isolated gill tissue is stimulated by the replacement of sodium in the medium with choline.

7. The data are consistent with a sodium-coupled mechanism for the transport of amino acid into gill tissue. However, energetic considerations cast doubt on the adequacy of such a mechanism to account fully for the observed trans-epidermal transport.


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